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Attorney Docket No.: 2798-1-001

IN THE CLAIMS:

Final Rejection

1. (currently amended) A method for producing 4-O- $\beta$ -D-galactopyranosyl-D-xylose enzymatically that comprises:
  - (i) preparing a first reaction mixture of 2-20% by weight of D-xylose ~~0-5-5-0.5~~ to 5% by weight of a  $\beta$ -D-galactopyranoside substrate 75-97.5% by weight of a reaction medium that comprises buffered water at a pH between 5.0 and 9.0; adding 10 to 1,000 units of a  $\beta$ -D-galactosidase enzyme, per gram of  $\beta$ -D-galactopyranoside, to the first reaction mixture; and obtaining a second reaction mixture
  - (ii) subjecting the second reaction mixture to a reaction at a temperature comprised between a temperature higher than the freezing point of the second reaction mixture and 45° C., for 2 to 48 hours, in order to form disaccharides in the second reaction mixture;
  - (iii) stopping the reaction when the disaccharides have been formed in the desired amount, by means of a treatment selected from the group consisting of deactivation of  $\beta$ -D-galactosidase by freezing the second reaction mixture at a temperature between -20 ° C. and -170 ° C., deactivation of  $\beta$ -D-galactosidase by heating the second reaction mixture at a temperature between 95 and 110 ° C., and separation of  $\beta$ -D-galactosidase from the second reaction mixture by ultrafiltration; obtaining a third reaction mixture;
  - (iv) separating an aglyconic fragment of the  $\beta$ -D-galactopyranoside substrate used in the first step from the third reaction mixture by extraction or filtration; obtaining a fourth reaction mixture;

(v) isolating fractions that contain 4-O- $\beta$ -D-galactopyranosyl-D-xylose, by a method selected from the group consisting of addition of celite to the fourth reaction mixture; followed by solid-liquid extraction with a solvent and elution with a first eluent in a column; and directly adding active carbon to the fourth reaction mixture followed by filtration and elution with a second eluent,

(vi) crystallizing the fractions that contain 4-O- $\beta$ -D-galactopyranosyl-D-xylose in a crystallization mixture selected among from the group consisting of mixtures of acetone/methanol in a ratio between 5/1 to 20/1 and mixtures of acetone/water in a ratio between 5/1 to 20/1.

2. (previously presented) The method according to claim 1, wherein the fourth reaction mixture is concentrated before being subjected to elution in the column.

3. (previously presented) The method according to claim 1, wherein the mixture of acetone/methanol has a ratio of 10/1.

4. (previously presented) The method according to claim 1, wherein the mixture of acetone/water has a ratio of 10/1.

5. (previously presented) The method according to claim 1, wherein the first eluent is a mixture of water/isopropanol that contains 1 to 10% (v/v) of isopropanol.

6. (previously presented) The method according to claim 1, wherein the mixture of water/isopropanol contains 2% (v/v) of isopropanol.

7. (previously presented) The method according to claim 1, wherein step (v) consists of adding celite to the fourth reaction mixture and concentrating to dryness, followed by solid-liquid extraction with an organic solvent in a Soxhlet extractor that has a cartridge made out of a material compatible with said solvent, and eluting with a first eluent in a column selected from

the group consisting of filtration columns with cross-linked dextrane polymer fillers, filtration columns with acrylamide polymer fillers, filtration columns of active carbon and active carbon-celite columns.

8. (previously presented) The method according to claim 7, wherein the solvent is ethyl acetate.

9. (previously presented) The method according to claim 7, wherein the solvent is used in an amount between 10 ml and 25 ml per gram of initial xylose.

10. (previously presented) The method according to claim 7, wherein the celite is used in an amount between 1 g and 2 g per gram of initial xylose.

11. (previously presented) The method according to claim 7, wherein the column is of active carbon-celite wherein the carbon is deactivated by adding 35% hydrochloric acid.

12. (previously presented) The method according to claim 11, wherein the celite is used in an amount between 0.5 g and 2 g of celite per gram of initial xylose.

13. (previously presented) The method according to claim 11, wherein the active carbon is used in an amount between 0.5 g and 2 g of active carbon per gram of initial xylose.

14. (previously presented) The method according to claim 7, wherein said first eluent is used in an amount between 5 ml and 25 ml per gram of initial xylose.

15. (previously presented) The method according to claim 11, wherein the hydrochloric acid is used in an amount between 0.5 ml and 1.5 ml per gram of initial xylose.

16. (previously presented) The method according to claim 1, wherein in step (v), the fourth reaction mixture is subjected to direct addition of at least a second eluent on the active carbon wherein the 4-O- $\beta$ -D-galactopyrano- syl-D-xylose is adsorbed on the active carbon and the second eluent is water followed by diluted isopropanol with a growing proportion in volume of

isopropanol in successive steps.

17. (previously presented) The method according to claim 16, wherein the proportion in volume of isopropanol is between 1% and 3% in a first step, between 3% and 5% in a second step and between 5% and 7% in a third step.

18. (previously presented) The method according to claim 16, wherein the active carbon is used in an amount between 2 g and 4 g of active carbon per gram of initial xylose.

19. (previously presented) The method according to claim 16, wherein the second eluent is used in a total amount between 30 ml and 50 ml of second eluent per gram of initial xylose.

20. (currently amended) The method according to claim 1, wherein the reaction is slowed stopped by cooling the second reaction mixture at 0 ° C.

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21. (previously presented) The method according to claim 1, wherein the fourth reaction mixture is obtained by separating the aglyconic fragment from the β-D-galactopyranoside substrate by means of filtration.

22. (previously presented) The method according to claim 1, wherein the proportion of D-xylose in the second reaction mixture is 7.5% by weight.

23. (previously presented) The method according to claim 1, wherein the proportion of β-D-galactopyranoside in the second reaction mixture is 1.5% by weight.

24. (previously presented) The method according to claim 1, wherein 20 units of β-D-galactosidase per gram of β-D-galactopyranoside are added.

25. (previously presented) The method according to claim 1, wherein the reaction medium also comprises at least a cosolvent medium selected from the group consisting of dimethylsulfoxide, dimethylformamide, dioxane and mixtures thereof.

26. (previously presented) The method according to claim 25, wherein the reaction medium comprises 20% by weight of the cosolvent medium.
27. (previously presented) The method according to claim 1, wherein the reaction is carried out at a constant temperature.
28. (previously presented) The method according to claim 1, wherein the reaction temperature is from -5 ° C. to 40 ° C.
29. (previously presented) The method according to claim 1, wherein the reaction temperature is higher than the freezing temperature of the second mixture and lower than 0 ° C.
30. (previously presented) The method according to claim 1, wherein the reaction temperature is -5 ° C.
31. (previously presented) The method according to claim 1, wherein the reaction temperature is room temperature.
32. (previously presented) The method according to claim 1, wherein the reaction medium is buffered to a pH of 7.
33. (previously presented) The method according to claim 1, wherein in step (iii), the reaction is stopped by freezing the second reaction mixture at a temperature of -78 ° C.
34. (previously presented) The method according to claim 1, wherein in step (iii), the reaction is stopped by heating the second reaction mixture up to a temperature of 100 ° C.
35. (previously presented) The method according to claim 1, wherein in step (iii), the reaction is stopped by separating the β-D-galactosidase by ultrafiltration.

36. (previously presented) The method according to claim 1, wherein the  $\beta$ -D-galactopyranoside substrate is selected from the group consisting of o-nitrophenyl  $\beta$ -D-galactopyranoside and lactose.

37. (previously presented) The method according to claim 1, wherein the  $\beta$ -D-galactosidase enzyme is *E. coli*  $\beta$ -D-galactosidase.

38. (previously presented) The method according to claim 1, wherein the  $\beta$ -D-galactosidase enzyme is *Kluyveromyces lactis*  $\beta$ -D-galactosidase.

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39. (previously presented) A 4-O- $\beta$ -D-galactopyranosyl-D-xylose obtained by the method of claim 1.

40. (original) A composition for in vivo evaluation of intestinal lactase in humans, characterized in that it comprises a 4-O- $\beta$ -D-galactopyranosyl-D-xylose obtained by means of the process defined in claim 1.

41. (original) A solution for the in vivo evaluation of intestinal lactase in humans, characterized in that it comprises a solution selected between aqueous solutions and saline solutions of a 4-O- $\beta$ -D-galactopyranosyl-D-xylose obtained by means of the process defined in claim 1.

42. (original) Use of 4-O- $\beta$ -D-galactopyranosyl-D-xylose prepared according to claim 1, in the preparation of a composition for in vivo evaluation of intestinal lactase in humans.

43. (original) Use of 4-O- $\beta$ -D-galactopyranosyl-D-xylose prepared according to claim 1, in the preparation of a solution selected between saline solutions and aqueous solutions for in vivo evaluation of intestinal lactase in humans.

44. (original) Use according to claim 42, characterized in that the 4-O- $\beta$ -D-galactopyranosyl-D-xylose is combined with pharmaceutically acceptable amounts of at least one additive selected

from among stabilizers, protecting agents, flavoring agents, lactose, gelling agents, fluidizing agents and preservatives.